

# A Review on Formulation, Characterization and Applications of Nanoemulsion

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DOI: <https://doi.org/10.38177/ajast.2024.8106>

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Article Received: 03 December 2023

Article Accepted: 22 February 2024

Article Published: 09 March 2024

## ABSTRACT

This review discusses the development, manufacturing, fabrication, and manipulation of Nanoemulsions, an advanced drug delivery method that addresses the limitations of conventional systems. Nanoemulsions are biphasic dispersions of immiscible liquids, either water in oil or oil in water, stabilized by an amphiphilic surfactant. They offer various drug delivery functionalities but face challenges in stability, structure control, and characterization. Nanoemulsions, with droplet sizes of 100 nm, are kinetically stable liquid-in-liquid dispersions with high surface area, robust stability, optical transparency, and tunable rheology. These submicron-sized emulsions are being studied for drug delivery and targeting, offering potential in cosmetics, diagnostics, drug therapies, and biotechnologies. They are used in cancer treatment, drug targeting, mucosal vaccines, transdermal drug delivery, lipophilic drugs, and self-nanoemulsifying drug delivery systems. This review explores various techniques for developing and characterizing Nanoemulsions, their formation and stability theories, and their current and future applications due to their unique structures and chemistries. This review discusses the importance of optimal formulation for nano-droplet systems, focusing on droplet size, solubilization, colloidal stability, optical and rheological properties. This research focuses on the study of various techniques of preparing Nanoemulsions i.e., high energy methods and low energy methods. This study explores the best methods for formulating Nanoemulsions, their characterization, release kinetics, and application in various fields.

**Keywords:** Nanoemulsion; High Energy Homogenization; Low Energy Homogenization; Drug Delivery System; Dispersion System; Rheology; Biphasic; Oral Mucosa; Characterization; Antiulcer.

## 1. Introduction

Nanoemulsion drug delivery systems are a promising tool for delivering and improving the bioavailability of hydrophobic drugs and bioactive food components in the blood. The majority of drugs are hydrophobic (lipophilic) in nature, thus leads to low solubility and bioavailability problems; the bioactive food components also show low bioavailabilities in conventional doses [1, 9, 34]. In the food industry, Nanoemulsions are being explored to encapsulate, stabilize, and deliver lipophilic constituents like flavours, omega-3 fatty acids, vitamins, preservatives, nutraceuticals. They have a number of potential advantages over conventional emulsions like incorporation into optically transparent products, may enhance the texture, stability, and bioavailability of products [2-5]. A widely used high energy method to reduce the droplet size of Nanoemulsions is ultrasonication. In this method, mechanical vibrations from ultrasound waves ( $> 20$  kHz) create sinusoidal pressure variation in the emulsion system [6, 7, 35]. The objective of this review are to explore various techniques for developing and characterizing Nanoemulsions, their formation and stability theories, and their current and future applications due to their unique structures and chemistries.

## 2. Formulation Techniques of Nanoemulsion

Nano emulsions are often produced in high-pressure homogenizers.

### 2.1. Construction

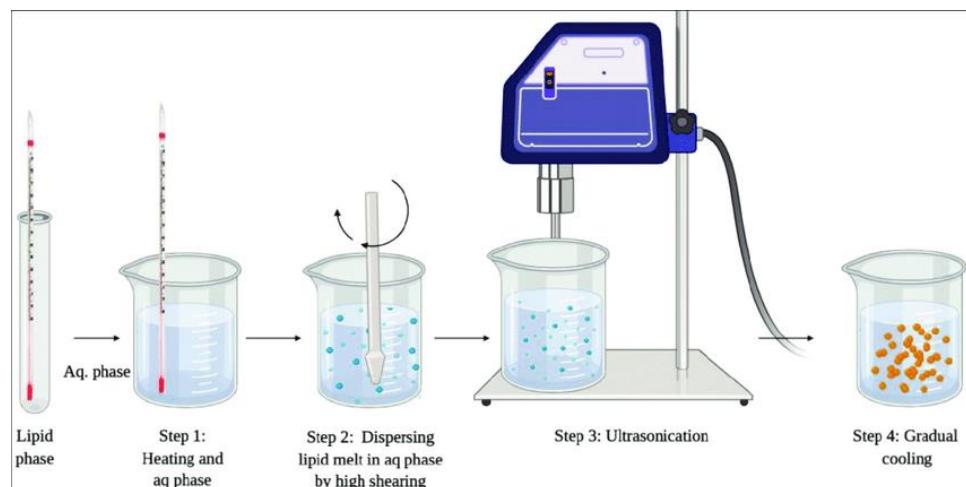
- A pump used in high-pressure homogenizers increases the dispersion's pressure by 500–5000 psi.
- The homogenizing valve's opening through which fluids are forced [1].

## 2.2. Process

A coarse emulsion is formed using a high shear mixer and introduced into a high-pressure homogenizer, resulting in a fine emulsion. Forces like turbulence, shear, cavitation, shock, shear stress, pressure gradient, and expansion shear cause droplet breakage [2]. The homogenizer employs various nozzle types to enhance droplet fracturing, result in a Nanoemulsion with a lipophilic core separated by a monomolecular phospholipid layer [3]. The final product undergoes hydraulic shear and turbulence, forming a small particle emulsion, effectively reducing the size of coarse emulsions by mixing oil and water separately.

## 2.3. Operational parameters

The droplet size decreases with increasing homogenization pressure, emulsifier adsorption rate and interfacial tension, and increases due to the decrease of these factors.



**Figure 1.** Formulation of Nanoemulsion using cold high-pressure homogenization technique [4]

## 2.4. Merits

- The homogenizer produces smaller particle sizes by delivering the power in the shortest possible time with the most homogeneous flow (up to 1 nm).
- High efficiency.

## 2.5. Demerits

- Heavy reliance on energy.
- An increase in emulsion temperature during processing.

## 2.6. Ultrasonication

Ultrasonication is a high-energy method used to decrease droplet size in emulsions by causing a sinusoidal pressure shift due to mechanical vibrations above 2 kHz [7].

### 2.6.1. Construction

A piezoelectric probe is used to produce a strong disruptive force by the help of its tip.

### 2.6.2. Process

The process involves mixing a homogenous oil phase into an aqueous phase, creating a coarse emulsion, and then subjecting it to ultrasonication to create Nanoemulsions. Bubbles are produced through cavitation, causing droplets to condense.

### 2.6.3. Merits

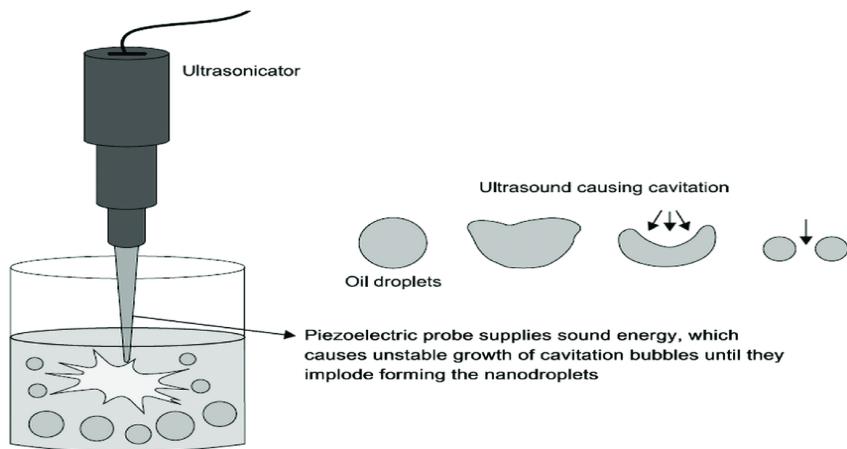
Less energy expenditure.

### 2.6.4. Demerits

Contamination caused by probe.

### 2.6.5. Operational parameters

Increased sonication time and input power decrease droplet size. Probe placement, depth, and contact with solid surfaces affect pressure distribution and wave reflection [8].



**Figure 2.** Ultrasonication technique [9]

### 2.6.6. Example of ultrasonication method

#### Preparation of a novel curcumin Nanoemulsion by ultrasonication

Curcumin Nanoemulsion (Cur-NE) was created using high-energy ultrasonication, dissolved in oil, combined with surfactants, and titrated with Milli-Q water to create a coarse emulsion. Using a 20 kHz ultrasonic processor, the final Nanoemulsion was produced with the following settings: 40.0% ultrasonication intensity, 10.0 minutes of ultrasonication time, and 50 °C temperature. The process was facilitated by cavitation, a high-intensity ultrasonication phenomenon, resulting in Cur-NE with improved Brownian motion and smaller globule sizes for extended storage [10].

### 2.7. Micro fluidization

the droplet size of the previously formed coarse emulsion is reduced by using micro fluidizers [11]. The working mechanism for size reduction includes hydraulic shear, impact, attrition, impingement, intense turbulence and cavitation [8].

### 2.7.1. Construction

A microfluidizer consists of

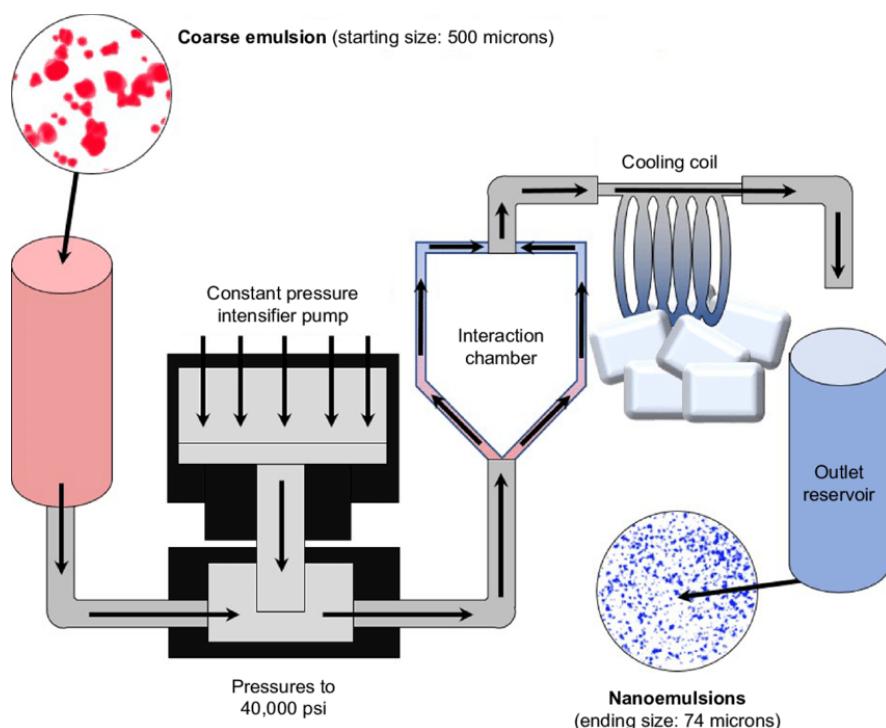
- An Inlet feed;
- A pressure intensifier pump;
- An interaction chambers;
- Cooling coil;
- Outlet.

### 2.7.2. Process

The pre-emulsion feed is divided into two channels using a stainless-steel block, which are positioned inside the device. The channel of the block gets smaller until it is about 75  $\mu\text{m}$  wide. The two feeds are made to collide directly. As a result, a very high shear is generated, resulting in the production of Nanoemulsion [11].

### 2.7.3. Operational parameters

The energy input can be increased by adjusting the operating pressure or emulsification time, which can be achieved by repeating the procedure multiple times [11].



**Figure 3.** Microfluidization Technique [12]

### 2.7.4. Merits

No contamination of feed material [8].

Continuous mass production of large quantities of goods and preparations is possible [13].

### 2.7.5. Demerits

- As the emulsification begins quickly, the biopolymers used in this method are unable to stabilize recently disrupted droplets in that short amount of time.
- High energy density that is produced during the procedure.

In "over-processing," the effects of droplet disruption are amplified by recoalescence, leading to an increase in EDS (Energy Density Spread), when the energy density is raised above the optimum level [11].

### 2.7.6. Example of microfluidization method

#### Production of sub-micron emulsions by ultrasound and microfluidization techniques

Previously prepared coarse emulsions (at room temperature) were passed through an air-driven microfluidizer (Model M-110 L, Microfluidics, USA). The system was fed pre-emulsion via a 200 ml glass reservoir. The device divides the pre-emulsion feed into two opposing channels in a stainless-steel block (a ceramic interaction chamber); these channels narrow to about 75 µm in width, and the two jets of pre-emulsion collide head-on at high pressure, resulting in extreme shear. When the air pressure at the regulator is 530 kPa, the typical pressure of the liquid jets flowing through the channels is about 120 MPa due to mechanical amplification of 232. The emulsions' volume flow rate was  $4 \times 10^{-6}$  m<sup>3</sup>/s 60 MPa for one cycle. The experiments were duplicated [11].

### 2.8. Phase inversion by temperature

The process involves heating surfactant, oil, and water, stirring until cool, to produce Nanoemulsions with small droplets and narrow particle sizes, ensuring high reliability and consistency [14].

#### 2.8.1. Working principle and mechanism

The PIT method uses temperature fluctuations to alter the hydration characteristics of nonionic surfactant head groups. At low temperatures, the head-group is highly hydrated, while at high temperatures, it becomes dehydrated. This affects the surfactant's water solubility and ideal surfactant monolayer curvature [14].

#### 2.8.2. Phase inversion temperature determination (PIT)

The hydrophilic-lipophilic balancing temperature (HLB) was calculated using electrical conductivity method. Mixtures of decane, water, and surfactant were agitated, and the HLB temperature, or phase inversion temperature, was determined by heating [15].

#### 2.8.3. Emulsification by phase inversion by temperature

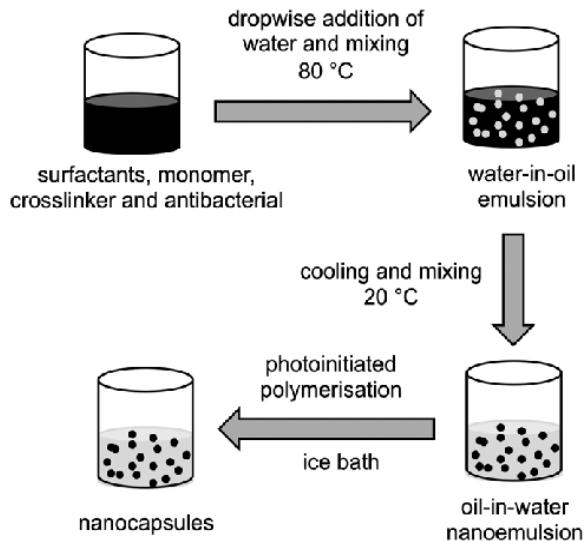
There are two steps involved. Initially, a temperature up to 15 °C higher than the PIT corresponding to the given surfactant concentration was achieved by simultaneously and separately heating the water phase and the oil phase containing the surfactant. When the oil phase reached that temperature, water was added, and the mixture was removed from the heat source and allowed to cool naturally before being transported to the PIT.

The oil phase was solubilized into a bicontinuous microemulsion at the HLB temperature, then chilled to 25 degrees Celsius and continuously stirred during chilling [15].

#### 2.8.4. Process variables

Surfactant concentrations affect the PIT, droplet size, particle morphology and storage stability.

Oil phase composition affects the turbidity which has direct impact on the PIT [14].



**Figure 4.** Mechanism of Nanoemulsion formation by PIT method [14]

#### 2.8.5. Merits

- It is simple to perform and implement.
- There is no need for sophisticated equipment.

#### 2.8.6. Demerits

- Higher surfactant concentrations cause instability problems.

#### 2.8.7. Example

##### Preparation of Cinnamon Nanoemulsion by phase inversion by temperature method

The ratio of cinnamon oil to MCT was changed from 0:10 to 1:9 to 2:8, 3:7 to 5:5, 6:4, 7:3, 8:2, and 10:0 while keeping the overall amounts of the oil phase (10 wt%), surfactant (10 wt%), and water phase (80 wt%) constant. Tween® 80 and deionised water were added after three minutes of blending cinnamon oil and MCT. The study involved combining components for 30 minutes, heating each solution to 15 °C, determining PIT for minimal turbidity, and cooling twice. Initially, cooling to the PIT temperature may result in the formation of a stable micro emulsion. The system was then rapidly cooled by adding 250 g of cold, deionised water (4 °C) and stirring continuously for three minutes. The study aimed to determine the mean droplet diameter, stability, and particle size distribution of each sample by rapidly cooling it to the PIT temperature [14].

#### 2.9. Solvent displacement method

This method involves combining the organic phase—which contains the oil dispersed in a solvent such as ethanol or acetone—with the aqueous phase, which contains the surfactants [16].

### 2.9.1. Working principle and mechanism

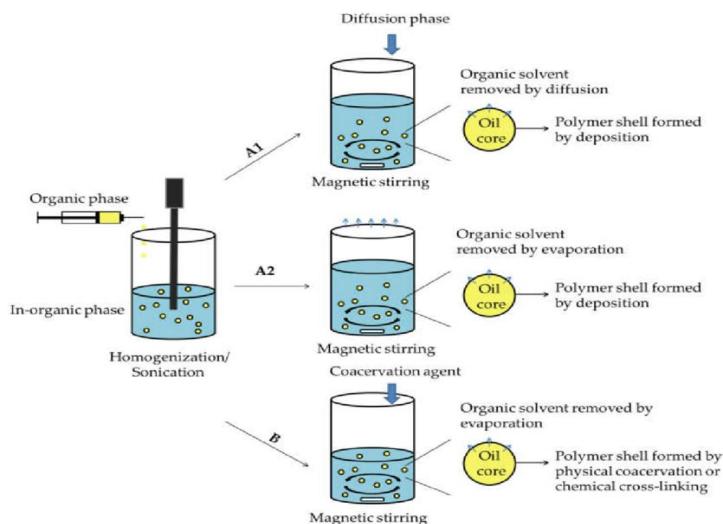
Emulsification occurs spontaneously due to diffusion of organic solvent, which may be removed later by vacuum evaporation [16].

### 2.9.2. Process

The solvent displacement method precipitates polymers from an organic solution, dissolves them in a semipolar solvent, and then adds it to an aqueous solution containing stabilizer, forming Nanoparticles instantly [17].

### 2.9.3. Process variables

The choice of emulsifying device is influenced by factors like volume, viscosity, surfactant type, temperature, and droplet size, with optimization of formulation parameters achieving desired Nanoemulsions [16].



**Figure 5.** Representation of Solvent Displacement Method [18]

### 2.9.4. Merits

- Requires no heating.
- No requirement of an organic solvent and LC or ME as a phase.

### 2.9.5. Demerits

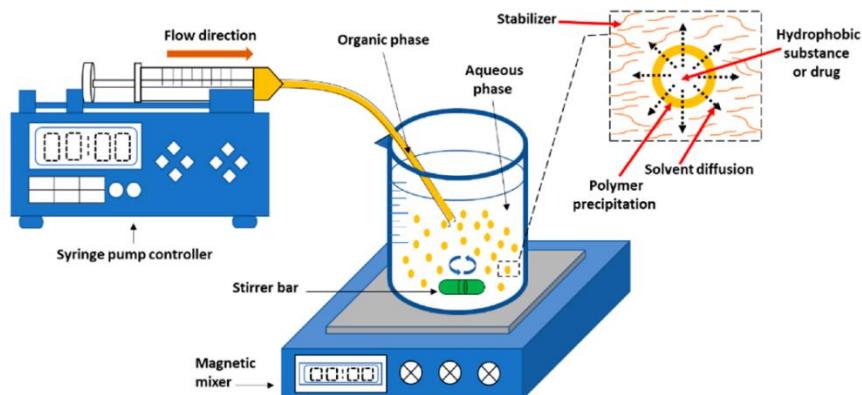
The need of a large ratio of solvent and oil for the production of small sized droplets of the disperse phase [16].

### 2.9.6. Example

#### Preparation of maltodextrin-stabilized $\alpha$ -tocopherol Nanoemulsions using the solvent-displacement method

The study created  $\alpha$ -tocopherol Nanoemulsions using solvent-displacement and different ratios of Polysorbate 20 and maltodextrin, using pure deionized water and diluted Polysorbate 20 with purified water.  $\alpha$ -tocopherol was dissolved in acetone at a concentration of 1% w/v to prepare the organic phase. For all samples, the organic-to-aqueous phase volume ratio was 1:1. The organic phase and Polysorbate 20 solution were slowly added to the maltodextrin solution under conventional homogenization at 15,000 rpm for 10 minutes after the solutions

had been homogenized using magnetic stirring. Finally, acetone was extracted from the system using a rotary evaporator set to 30 °C for 20 minutes [18].



**Figure 6.** The nanoprecipitation process is illustrated in a diagram [20]

## 2.10. Self-Emulsifying Nanoemulsion (SNEDDS)

SNEDDS are isotropic mixtures of oil, surfactant, co-surfactant, and drug that form aqueous oil-in-water emulsion with little stirring [19].

### 2.10.1. Process and mechanism

SEFs are mixtures of oil, surfactant, co-surfactant, and co-solvents that form a transparent, isotropic solution that emulsifies under gentle agitation, similar to the gastrointestinal tract (GIT).

### 2.10.2. Variables

There are many factors that affect SNEDDS:

High-dose drugs are not suitable for SNEDDS due to their limited water solubility and difficulty in delivering lipids. SNEDDS solubility in the oily phase is crucial, and larger surfactant or co-surfactant roles can lead to precipitation.

### 2.10.3. Merits

- Increased consistency in drug absorption.
- Drug(s) are selectively targeted toward a specific absorption window in the GI tract.
- Drug(s) are shielded from the gut environment.
- Delivery profile management.
- Variability has been reduced, including food effects.
- Increased oral bioavailability allows for dose reduction and high drug loading efficiency.
- Low production costs.
- Increased stability.

## 2.10.4. Demerits

- There are no good predictive in vitro models for evaluating formulations.
- Traditional dissolution methods are ineffective because formulations are not dependent on digestion prior to drug release.
- Further development and validation of the in vitro model is required. Various lipid-based prototype formulations must be developed and tested in vivo.
- GIT may be irritated by chemical instabilities of drugs and high surfactant concentrations in formulations (approximately 30-60%).
- Volatile co-solvents may migrate into the shells of soft or hard gelatin capsules, causing lipophilic drugs to precipitate.
- The dilution effect of the hydrophilic solvent may increase the precipitation tendency of the drug. Validation of multi-component formulations becomes more difficult.

## 2.10.5. Example of self-emulsifying Nanoemulsion

### **Self-Nanoemulsifying Drug Delivery System of chlorpromazine**

Chlorpromazine ( $\log P = 5.6$ , aqueous solubility 2.55 mg/L) is one of the most important and effective anti-psychotic and anti-emetic drugs in the Phenothiazine derivative class. Chlorpromazine, classified as class IV in the Biopharmaceutical classification system (BCS), is a dopamine antagonist with low oral bioavailability due to variable oral bioavailability and significant first-pass metabolism. To improve bioavailability, it should be packaged with a delivery system [20].

## 3. Characterization of Nanoemulsions

### **3.1. Droplet size**

#### **Transmission electron microscopy**

To investigate form and size of nanoparticles transmission electron microscopy is used. For this we use 300 mesh copper/carbon Transmission electron microscopy (TEM) grid with glow discharge. Samples are prepared by incorporating dilute solutions and then drying at room temperature. For TEM only most stable emulsions can be utilized [19]. The specimens were positioned on a polycarbonate base, and any surplus water was allowed to evaporate naturally at room temperature ( $25 \pm 8^\circ\text{C}$ ). Subsequently, they undergo drying in a critical point dryer utilizing carbon dioxide, followed by sputter coating with gold using a metallizer. Finally, the samples were scrutinized using a scanning electron microscope with an operational accelerating voltage of 20 kV.

### **3.2. Interfacial tension**

Interfacial tension is used to study formulation and characteristics of Nanoemulsions. When surfactant phase is in equilibrium with oil and aqueous phases, ultralow levels of interfacial tension are indicated with phase behaviour. Extremely low levels of interfacial tension can be determined by spinning drop equipment [28].

Nanoemulsions droplet size is measured by photon correlation spectroscopy. It is done by using a volumetric flask in which 0.1ml formulation and 50ml water is added and mixed by inverting flask gently. Measurements are taken by setting zeta sizer and light scattering monitor at 25 °C at specific angle (90° or 180°) [23].

### 3.3. Zeta potential

Zeta potential is a measure of particle charge which is important characteristic to determine stability of Nanoemulsions. High zeta potential indicates stability which means solution show resistance to aggregation. Low potential indicates attraction exceeds repulsion and dispersion flocculates. This measure indicates forces between particles at Nanoemulsions surface which helps in stabilization of Nanoemulsions. For electrostatically stable emulsions zeta potential must be 30 mV. For nano scaled particles zeta potential influenced by manufacturer such as particle source, electrolyte concentration, pH, hydration, particle morphology [21].

Zeta potential was determined by using the Electrophoretic mobility of particles in an electric field using Zeta sizer Nano ZS Apparatus. Zeta potential of the formulation was measured using Beckman Coulter Delsa Nano C Particle analyzer, USA. Through the determination of the electrostatic magnitude and the repulsion or attraction charge between particles, the potential value of zeta gives an indication of the stability of the Nanoemulsion. To maintain stability, an emulsion has to attain a minimum of 30 mV (positive or negative) of zeta-potential value [25].

### 3.4. Refractive index

Refractive index is the net value of the components of Nanoemulsion and indicates the isotropic nature of formulation. It is the technique for assessing whether formulation is transparent or not also thermodynamic stability analysis of sample [28].

It is determined by putting sample drop on slide and then comparing with water having RI 1. 33 using refractrometer. If comparison of system's RI is relatable to water's RI, then formulation is transparent. Refractive index was determined by using refractometer [23].

### 3.5. Conductance

Conductometer is used to determine conductance of sample i.e., Nanoemulsions. An EC Tester 11+, USA conductance meter was used to test the electrical conductance of the Nanoemulsion at 25 degrees. Three runs of this test were conducted to ensure uniformity [26].

In this method electrodes immersed in emulsion system which is supplied with electric source and lamp. If o/w type emulsions then water conducts current and lamp lights up due to flow of current between electrodes. In case if oil is exterior phase so lamp is dark because emulsion is absent [23].

### 3.6. Viscosity

It is key characteristic of Nanoemulsion. The resistance to flow of fluids is termed as viscosity, or the friction that exists within fluids. The most frequently employed instrument to measure viscosity is Brookfield viscometer.

The viscosity of the produced Nanoemulsion was measured using the Brookfield DV-II+ Pro viscometer at 25 °C without dilution by taking average of three data points at specific shear rate. After the mixture had been in the

beaker for five minutes, spindle readings were taken at 0.5, 1, 2.5, and 5 rpm at maintained temperature and at room temperature for 12 weeks which suggests that the lower the storage temperature, there is increase in viscosity of Nanoemulsions. The viscometer's accompanying dial was read and recorded.

According to power law model, emulsions exhibit shear thinning behaviour under shear rate. It gives three ranges for n (flow behaviour index): n<1 for shear thinning fluid, n=1 for Newtonian fluids and n>1 for shear thickening fluids. An emulsion shows less than 1 n value [26].

$$\text{Viscosity} = \text{mass/volume} \quad (1)$$

The viscosity of Nanoemulsions can be determined at various shear speeds [23].

### 3.7. Dye test

Microscopic analysis is done for clear understanding. If o/w emulsions type then it continuously absorbs water soluble dye. Conversely, if w/o emulsion it only uptakes water soluble dye in dispersion phase and the colour is not uniform [23].

### 3.8. Creaming test

Following homogenization, 10 milliliters of the Nanoemulsions were immediately put into a test tube, firmly sealed, and kept at room temperature ( $25 \pm 2^\circ\text{C}$ ) for seven days.

The creaming stability was determined by visually examining and then calculating the creaming index percent

$$\text{Creaming index percent} = (\text{HL}/\text{HE}) \times 100\% \quad (2)$$

where HL is the entire height of the cream layer and HE is the overall height of the emulsions [34]. Increased creaming index indicates the presence of emulsion instability, which can be attributed to flocculation, aggregation, coalescence, or high particle size [25].

### 3.9. pH values and total soluble solid content

At  $-20^\circ\text{C}$ , the total soluble solid content was measured in Brix is the result of triplicate measurements made with a Pocket Pal-1 refractometer. The pH was calculated using the pH meter by submerging the instrument bulb into 30 ml of each produced formulation [25].

### 3.10. Texture Analysis

The Nanoemulsion samples were kept in plastic containers at  $-20^\circ\text{C}$  for a whole day in order to conduct the texture analysis. At room temperature ( $25 \pm 2^\circ\text{C}$ ), measurements of firmness, hardness, consistency, cohesiveness, and viscosity index were made using a texture analyzer TAXT2i fitted with a 2-mm-diameter acrylic cylindrical probe. The samples' geometrical centers had a penetration depth of 10 mm, and their penetration rates were 1 mm/s.

After being hardened at  $-30^\circ\text{C}$ , the Nanoemulsion was sliced to fit into a tiny cylindrical cup with a diameter of 4.5 cm and a depth of 30 mm. It was then tempered overnight to  $-15^\circ\text{C}$  in preparation for the analysis. The penetration speed of the probe was 2 mm/s up to a 20 mm distance [25].

### 3.11. Creaming and cracking

Each multiple Nanoemulsion (MNE) was sampled in 30 ml and placed in a glass bottle with a screw lid (height 65 mm and inner diameter 25 mm). The container was then allowed to stand at  $25 \pm 2$  °C for a day before being checked for physical changes. The permanent/irreversible division or separation of the internal/dispersed phase (where oil and water are clearly separated) at the top of the emulsion is known as cracking, which is a physical instability. The given Equation was used to calculate the cream layer height (top layer) and the overall emulsion height in the event that the emulsions are divided into cream and serum layers. This allowed for the determination of the percentage of creaming.

$$\text{Creaming (\%)} [28] = 100 \times \frac{\text{Height of cream layer}}{\text{Total height of emulsion}} [28] \quad (3)$$

### 3.12. Entrapment efficiency

To determine the percentage drug encapsulation efficiency, the concentration of unentrapped drug, or free drug, in the formulation was assessed. This concerns since it has an impact on the medication molecule's release characteristics. Equation was used to determine the quantity of drug encapsulated per unit weight of formulation after the entrapped drug was separated from the Nanoemulsion formulation.

$$\% \text{EE} = (\text{amount of drug added} - \text{free (unentrapped) drug}) / (\text{amount of drug added}) \times 100 [28] \quad (4)$$

### 3.13. Differential Scanning Calorimetry (DSC)

It is a thermo analytical technique which measures difference in amount of heat needed to increase temperature of sample and reference. Both reference and sample are maintained at same temperature throughout experiment. The sample should have well defined heat capacity over the range of temperature. This technique is employed to detect phase transitions as melting of crystalline agents and analyze proportion of solid fat or ice crystals in emulsion. It is also used to detect crystallization temperature of mixture of surfactants [21].

### 3.14. Fourier Transform Infrared Spectroscopy (FTIR)

It is based on infrared radiations that are absorbed by sample. It gives spectrum that represent molecular absorption and transmission forming molecular finger print of sample. This fingerprint represents characteristic absorption peaks corresponding to frequencies of vibration between atoms of material. The size of peaks in spectrum is direct indication of amount of material in sample. Advantage of FTIR is to determine amount of component in mixture and to determine quality and consistency of sample. It gives accurate and reproducible measurements [21].

### 3.15. In vitro dissolution profile

#### 3.15.1. United State Pharmacopoeia (USP) type-II apparatus

A dissolution apparatus type II was used for a drug release investigation. 900 milliliters of pH 1.2 simulated gastric fluids served as the dissolving medium. Every Nanoemulsion formulation has gone through this investigation at various pH levels by being placed in a dialysis membrane bag and replaced with fresh medium. At certain intervals, a 5 ml sample was taken and replaced with new media. Each sample was examined to determine drug concentration using a UV-Vis spectrophotometer set to analyze at lambda max of 210nm after being filtered with a syringe filter

of 0.45 µm. Then samples are analyzed in HPLC or by other methods for determining release behaviour which is then compared with standards [27].

### 3.15.2. Franz cell apparatus method

The Franz cell device with a diffusion area of 1.79 cm<sup>2</sup> and a receiver chamber volume of 16 ml was used for in vitro drug release. A synthetic cellulose acetate membrane (Merck, Brazil) was used, which had been previously moistened. The donor and receiver compartments were then separated by the membrane. To maintain sink conditions, the receiver chamber was filled with a physiological solution containing 1% -cyclodextrin as a solubilizer (maximum MS solubility = 2.13 mg/ml). A Peltier-Type Temperature Control Equipment was used to keep the receptor compartment at 32 °C by employing an external thermal bath. Throughout the experiment, continuous stirring was maintained. Bubbles were avoided by sonicating the receiver solution before to the experiment.

On top of the membrane, the equivalent of 10 mg of formulation was deposited. The donor compartment was secured to the receiving compartment, which was then sealed with Parafilm®. Aliquots of 500:1 was collected after 30 minutes, 1, 2, 3, 4, 6, 7, 8, and 24 hours. An equivalent volume of new medium was poured to maintain the washbasin condition. A validated HPLC method was used to find the concentration of MS in each sample [28].

### 3.15.3. Membrane diffusion method

Drug release studies were carried at temperature 32 °C by using (standard regenerated cellulose and Spectra). For Experiment of release take 5g of Formulation along with the receptor solution filled in dialysis chamber for 24 hours. By using UV-Visible Spectroscopy the concentration of drug in receptor solution was analyzed by wavelength in 350 nm. Surfactant was added in solution to increase solubility in receptor solution. The receptor solution consists of 1.5% w/w polysorbate buffer at pH (7.4) [29].

## 4. Applications

### 4.1. Nanoemulsions in drug delivery

Nanoemulsions are utilized in various drug delivery methods, including topical, ocular, intravenous, intamuscular, internasal, and oral delivery. They utilize their lipophilic nature to dissolve water-insoluble drugs and their tunable charge and rheology to create aqueous solutions. Nanoemulsions also offer advantages for hydrophobic drugs, and are used as ultrasound imaging agents [30].

### 4.2. Oral Delivery

Lipids can be used as Nanoemulsions to increase drug absorption in the GIT, particularly protein drugs, by loading them inside lipids, thereby enhancing the overall absorption process [31].

### 4.3. Topical delivery

Enhancing the permeation of drugs for topical application is challenging due to poor dispersibility and skin irritant effects. Nanoemulsions, such as soybean lecithin, tween, and poloxamer, offer a combination of penetration enhancement and concentration gradient.

#### 4.4. Intravenous delivery

Parenteral Nanoemulsions deliver drugs with lower bioavailability and narrow therapeutic indices, converting into stealth Nanoemulsions by coating or attaching hydrophilic moiety, enhancing permeability and retention for tumor targeting.

#### 4.5. Targeted drug delivery

Nanoemulsion technology has been widely utilized as transdermal drug delivery system. Nanomaterials' small size allows them to connect more substances due to their large surface area and easy transport, and their surface drainage allows them to accumulate at skin level [33].

#### 4.6. Nanoemulsion in cosmetics

Newer materials (NEs) are increasingly important for controlled cosmetic delivery and optimized dispersion of active ingredients in skin layers. They are suitable for transporting lipophilic compounds and support skin penetration, increasing active ingredient concentration. NEs also have bioactive effects, reducing trans-epidermal water loss and strengthening skin barrier function. They are acceptable in cosmetics due to their lack of creaming, sedimentation, flocculation, and coalescence [10]. TRI-K Industries and Kemira have developed a new nano-based gel, Kemira NanoGel, to improve the effectiveness of skincare products. The unique NE Carrier system creates submicron emulsions from an oil-in-water concentrate, minimizing trans epidermal water loss and enhancing skin production. This technology is particularly useful in sun care, moisturizing, and anti-aging creams, and provides a good skin feel [36].

#### 4.7. Nanoemulsion in cell culture technology

Cell cultures are used for in vitro assays and producing biological compounds. Oil-soluble substances have been difficult to absorb by cells. New encapsulated substances (NEs) are a new method for delivering oil-soluble substances to mammalian cell cultures. These transparent, phospholipid-stabilized NEs have high bioavailability, improving cell growth and vitality, and allowing for toxicity studies of oil-soluble drugs in cell cultures.

#### 4.8. Nanoemulsion as an antimicrobial preparation

NEs are increasingly important for controlled cosmetic delivery and optimized dispersion of active ingredients in skin layers. They support skin penetration, increase active ingredient concentration, and have a small droplet size. NEs also have bioactive effects, reducing trans-epidermal water loss and avoiding creaming, sedimentation, flocculation, and coalescence compared to macroemulsion [10]. TRI-K Industries and Kemira have developed a new nano-based gel, Kemira NanoGel, to improve the effectiveness of skincare products. The unique NE Carrier system creates submicron emulsions from an oil-in-water concentrate, minimizing water loss and enhancing skin production. This technology is particularly useful in sun care, moisturizing, and anti-aging creams.

#### 4.9. Nanoemulsion to improve the per-oral delivery of poorly soluble drugs

Nanoemulsion has wide application in improving solubility of poorly soluble drugs. BCS class II and IV drugs, which are poorly soluble in water, face challenges in conventional dosage forms. Nanoemulsions offer a solution for improved solubility and therapeutic efficacy [32].

## 5. Conclusion

This study explores various Nanoemulsion formulating techniques, preparation, characterization, release studies, and kinetic modelling. This document discusses various high- and low-energy techniques such as high-pressure homogenization, ultrasonication, microfluidization, phase inversion by temperature, solvent displacement method, and self-emulsifying Nanoemulsion, respectively, that are considered the best methods for formulating Nanoemulsion. All Nanoemulsion formulations are generally considered effective, safe, and have improved bioavailability. Nanoemulsions offer advantages in drug delivery, masking oily liquids' unpleasant taste, and protecting drugs from hydrolysis and oxidation, making them popular for targeted anticancer, photosensitivity, and therapeutic agents. Moreover, various applications of Nanoemulsion are also being discussed, including the use of nanomeulsion in drug delivery systems like oral, topical, intravenous, intramuscular, intranasal, pulmonary, and ocular. Nanoemulsion also has broad applications in cosmetics, cell culture technology, and antimicrobial preparation to improve the per-oral delivery of poorly soluble drugs in transdermal drug delivery systems.

This novel method can be developed to overcome the drug limitations such as poor solubility, absorption, and miscibility with lipoidal cell membrane. Niosomes are adaptable to all delivery methods, so they can be used in a broad range of disciplines such as biotechnology, cosmetics and Pharmaceuticals etc. Niosomes will be an emerging novel technology for improving the bioavailability of drugs. It will be utilized to encapsulated wide range of bioactive compounds in Pharmaceutical, cosmetic and food industry.

### Declarations

#### Source of Funding

The study has not received any funds from any organization.

#### Competing Interests Statement

The authors have declared no competing interests.

#### Consent for Publication

The authors declare that they consented to the publication of this study.

### References

- [1] Borthakur, P., et al. (2016). Nanoemulsion: Preparation and its application in food industry. Elsevier, 3: 153–191. doi: 10.1016/B978-0-12-804306-6.00005-2.
- [2] Goindi, S., et al. (2016). Nanoemulsions: an emerging technology in the food industry, in Emulsions. Elsevier, Pages 651–688. doi: 10.1016/B978-0-12-804306-6.00019-2.
- [3] Bhatt, P., & Madhav, S. (2011). A detailed review on Nanoemulsion drug delivery system. International Journal of Pharmaceutical Sciences and Research, 2(10): 2482. doi: [http://dx.doi.org/10.13040/IJPSR.0975-8232.2\(10\).2482-89](http://dx.doi.org/10.13040/IJPSR.0975-8232.2(10).2482-89).

- [4] Yukuyama, M., et al. (2016). Nanoemulsion: process selection and application in cosmetics—a review. *International Journal of Cosmetic Science*, 38(1): 13–24. doi: 10.1111/ics.12260.
- [5] Zahi, M.R., et al. (2014). Formation and stability of d-limonene organogel-based Nanoemulsion prepared by a high-pressure homogenizer. *J. of Agricultural and Food Chemistry*, 62(52): 12563–12569. doi: 10.1021/jf5032108.
- [6] Krahn, C.L., et al. (2012). Isoflurane-loaded Nanoemulsion prepared by high-pressure homogenization: investigation of stability and dose reduction in general anesthesia. *Journal of Biomedical Nanotechnology*, 8(5): 849–858. doi: 10.1166/jbn.2012.1449.
- [7] Pratap-Singh, A., et al. (2021). Optimal ultrasonication process time remains constant for a specific Nanoemulsion size reduction system. *Scientific Reports*, 11(1): 9241.
- [8] Singh, Y., et al. (2017). Nanoemulsion: Concepts, development and applications in drug delivery. *Journal of controlled release*, 252: 28–49. doi: <https://doi.org/10.1016/j.jconrel.2017.03.008>.
- [9] Kumar, M., et al. (2019). Techniques for formulation of Nanoemulsion drug delivery system: a review. *Preventive Nutrition and Food Science*, 24(3): 225. doi: 10.3746/pnf.2019.24.3.225.
- [10] Ahmad N., et al. (2019). Preparation of a novel curcumin Nanoemulsion by ultrasonication and its comparative effects in wound healing and the treatment of inflammation. *RSC Advances*, 9(35): 20192–20206. doi: <https://doi.org/10.1039/C9RA03102B>.
- [11] Jafari, S.M., Y. He & Bhandari, B. (2007). Production of sub-micron emulsions by ultrasound and microfluidization techniques. *Journal of Food Engineering*, 82(4): 478–488. doi: 10.1016/j.foodeng.2007.03.007.
- [12] Ganesan, P., et al. (2018). Microfluidization trends in the development of systems and applications in chronic disease treatments. *International Journal of Nanomedicine*, 9(13): 6109–6121. doi: 10.2147/IJN.S178077.
- [13] Vuillemand, J.C. (1991). Recent advances in the large-scale production of lipid vesicles for use in food products: microfluidization. *Journal of Microencapsulation*, 8(4): 547–562. doi: 10.3109/02652049109021878.
- [14] Chuesiang, P., et al. (2018). Optimization of cinnamon oil Nanoemulsions using phase inversion temperature method: Impact of oil phase composition and surfactant concentration. *Journal of Colloid and Interface Science*, 514: 208–216. doi: 10.1016/j.jcis.2017.11.084.
- [15] Ee, S.L., et al. (2008). Droplet size and stability of nano-emulsions produced by the temperature phase inversion method. *Chemical Engineering Journal*, 140(1–3): 626–631. doi: 10.1016/j.cej.2007.12.016.
- [16] Jasmina, H., et al. (2017). Preparation of Nanoemulsions by high-energy and low energy emulsification methods in CMBEBIH 2017: Proceedings of the International Conference on Medical and Biological Engineering, Springer. doi: 10.1007/978-981-10-4166-2\_48.
- [17] Pathak, C., Vaidya, F.U., & Pandey, S.M. (2019). Mechanism for development of nanobased drug delivery system. *Applications of Targeted Nano Drugs and Delivery Systems*, Pages 35–67. doi: 10.1016/B978-0-12-814029-1.00003-X.

- [18] Firoozy, M., & Anarjan, N. (2019). Preparation of maltodextrin stabilized  $\alpha$ -tocopherol Nanoemulsions using solvent-displacement technique. *Food Science and Technology International*, 25(5): 404–413. doi: 10.1177/1082013219825893.
- [19] Uppulurj, K.B. (2015). Self nano emulsifying drug delivery systems for oral delivery of hydrophobic drugs. *Biomedical and Pharmacology Journal*, 6(2): 355–362. doi: <https://dx.doi.org/10.13005/bpj/425>.
- [20] Baloch, J., et al. (2019). Self-nanoemulsifying drug delivery system (SNEDDS) for oral bioavailability of chlorpromazine: in vitro and in vivo evaluation. *Medicina*, 55(5): 210. doi: 10.3390/medicina55050210.
- [21] Silva, H.D., Cerqueira, M.A., & Vicente, A.A. (2012). Nanoemulsions for food applications: development and characterization. *Food and Bioprocess Technology*, 5: 854–867. doi: 10.1007/s11947-011-0683-7.
- [22] Pathan, I., Mangle, M., & Bairagi, S. (2016). Design and characterization of Nanoemulsion for transdermal delivery of meloxicam. *Analytical Chemistry Letters*, 6(3): 286–295. doi: 10.1080/22297928.2016.1209126.
- [23] Martins, J.T., et al. (2018). Protein-based structures for food applications: From macro to nanoscale. *Frontiers in Sustainable Food Systems*, 2: 77. doi: <https://doi.org/10.3389/fsufs.2018.00077>.
- [24] Gurpreet, K., & Singh, S. (2018). Review of Nanoemulsion formulation and characterization techniques. *Indian Journal of Pharmaceutical Sciences*, 80(5). doi: 10.4172/pharmaceutical-sciences.1000422.
- [25] Mohammed, N.K., Muhiadin, B.J., & Meor Hussin, A.S. (2020). Characterization of Nanoemulsion of Nigella sativa oil and its application in ice cream. *Food Science & Nutrition*, 8(6): 2608–2618. doi: <https://doi.org/10.1002/fsn3.1500>.
- [26] Alam, A., et al. (2022). Babchi Oil-Based Nanoemulsion Hydrogel for the Management of Psoriasis: A Novel Energy Economic Approach Employing Biosurfactants. *Gels*, 8(12): 761. doi: 10.3390/gels8120761.
- [27] Alhamdany, A.T., Saeed, A.M., & Alaayedi, M. (2021). Nanoemulsion and solid Nanoemulsion for improving oral delivery of a breast cancer drug: Formulation, evaluation, and a comparison study. *Saudi Pharmaceutical Journal*, 29(11): 1278–1288. doi: 10.1016/j.jpsps.2021.09.016.
- [28] Malik, M.R., et al. (2022). Formulation and characterization of chitosan-decorated multiple Nanoemulsion for topical delivery in vitro and ex vivo. *Molecules*, 27(10): 3183. doi: <https://www.mdpi.com/1420-3049/27/10/3183>.
- [29] Miastkowska, M., et al. (2016). The kinetic study of isotretinoin release from Nanoemulsion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 510: 63–68.
- [30] Gupta A., et al. (2016). Nanoemulsions: formation, properties and applications. *Soft Matter*, 12(11): 2826–2841. doi: 10.1016/j.colsurfa.2016.07.060.
- [31] Pathak, K., Pattnaik, S., & Swain, K. (2018). Application of Nanoemulsions in drug delivery. In *Nanoemulsions*, Elsevier, Pages 415–433. doi: 10.1016/B978-0-12-811838-2.00013-8.
- [32] Thakur, A., Walia, M.K., & Kumar, S. (2013). Nanoemulsion in enhancement of bioavailability of poorly soluble drugs: a review. *Pharmacophore*, 4(1): 15–25.

- [33] Wu, Y., et al. (2013). The application of Nanoemulsion in dermatology: an overview. *Journal of Drug Targeting*, 21(4): 321–327. doi: 10.3109/1061186X.2013.765442.
- [34] Tayal, S., Sharma, A., & Kumar, A. (2023). Development and Characterization of Non-Aqueous based Self Emulsifying Nano Emulsion of Curcumin. *Middle East Journal of Applied Science & Technology*, 6(2): 144–152. doi: <https://doi.org/10.46431/MEJAST.2023.6216>.
- [35] Gupta, A., Eral, H.B., Hatton, T.A., & Doyle, P.S. (2016). Controlling and predicting droplet size of Nanoemulsions: scaling relations with experimental validation. *Soft Matter*, 12(5): 1452–8. doi: <https://doi.org/10.1039/C5SM02051D>.
- [36] Karthik, P., Ezhilarasi, P.N., & Anandharamakrishnan, C. (2017). Challenges associated in stability of food grade Nanoemulsions. *Critical Reviews in Food Science and Nutrition*, 57(7): 1435–50. doi: 10.1080/10408398.2015.1006767.